bio-protocol Preprint

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Electrophysiology and pharmacology for PHP

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An abbreviated version of this protocol was published in eLIFE in Jun 2019

Maintenance of homeostatic plasticity at the *Drosophila* neuromuscular synapse requires continuous IP_3-directed signaling

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Detailed protocol

1. In advance, prepare modified HL3 solution (70 mM NaCl, 5 mM KCl, 5 mM HEPES, 10 mM NaHCO3, 115 mM sucrose, 0.5 mM $CaCl_2$ (unless otherwise noted), 10 mM $MgCl_2$, 4.2 mM trehalose, pH 7.2). On the day of recording, prepare a 50 mL conical of it. This 50 mL conical is your stock saline.

2. Also in advance, prepare a 4 mM stock solution of Philanthotoxin-433 (PhTox). Freeze in 5 µL aliquots.

3. On the day of recording, thaw one 5 µL aliquot of 4 mM PhTox and dilute into 995 µL of HL3 stock saline. This 1:200 dilution yields 1000 µL PhTox saline with a working concentration of 20 µM PhTox.

4. Pick a wandering third instar Drosophila melanogaster larva for recording. Immerse in ~ 250 µL stock saline for dissection.

5. Working quickly, with the dorsal side up: pin the tail, then the head of the larva. Take care not to stretch out the animal along the anterior/posterior (AP) axis.

6. Using fine dissection scissors, make a small, shallow access incision on the dorsal side of the larva, near its tail, perpendicular to the AP axis. Take care not to unpin the larva.

7. Starting at the access incision, make a second shallow incision along the entire AP axis, going as far anterior as possible. Take care not to unpin the larva; keep it unstretched.

8. Aspirate away all of the stock saline used for dissection. Replace with ~ 125 µL PhTox saline, completely immersing the larva. Incubate in PhTox saline for 10-15 minutes. This incubation period will allow the NMJ to induce and express presynaptic homeostatic potentiation (PHP).

9. Aspirate away the PhTox saline and dispose of it properly. Replace with stock saline to finish the dissection.

NOTES: (a) You may now stretch the animal out if it facilitates your dissection, but it is not required. The first author of this study (TDJ) left preps unstretched and was able to garner high quality recordings. (b) Since PhTox will bind to muscle glutamate receptors during the incubation, there is no concern that the PHP induction will be reversed once you wash it off. PHP is successfully induced an expressed in the continuous presence of PhTox – or if it is washed off after 10 minutes.

10. With one hand, use forceps to carefully grip the larva's posterior trachea. With your other hand, hold your dissection scissors. Using both hands, gently lift trachea (forceps) and snip (scissors), working posterior to anterior. If you do this correctly, you will complete most of the dissection, removing the guts, fat, and trachea, without damaging muscles or nerves.

11. If you have left the central nervous system in the larva, grab it with the forceps and sever its segmental nerves with the scissors, taking care not to yank it hard or to damage the nearby body wall muscles.

12. Use four more pins to pin open the larva in a hexagonal fillet. Immerse in fresh stock saline. Move this fillet to the electrophysiology rig for recording.

13. Conduct sharp electrode electrophysiology using standard methods. We accept NMJs with muscle resting potentials of -60 mV or lower and an input resistances of 5 MΩ or greater.

14. Record spontaneous miniature excitatory postsynaptic potentials (mEPSPs) for 1-2 minutes. PhTox incubation should significantly reduce mEPSP amplitude compared to control mock incubation.

15. To record evoked potentials (EPSPs, also known as EJPs), suck the correct segmental nerve into your stimulus electrode. Starting with zero current on your stimulator, pass positive current through the nerve until just enough current excites both the Type-1b and Type-1s neurons in the segmental nerve yielding a full value EPSP. We use a 1 ms stimulus duration and stimulate 1 Hz for at least 30 recorded pulses. We know that some groups prefer a lower stimulus frequency (0.2-0.5 Hz), but in our hands the results are comparable.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Frank, C. A. and James, T. D.(2020). Electrophysiology and pharmacology for PHP. Bio-protocol Preprint. bio-protocol.org/prep331.
2. James, T. D., Zwiefelhofer, D. J. and Frank, C. A.(2019). Maintenance of homeostatic plasticity at the *Drosophila* neuromuscular synapse requires continuous IP_3-directed signaling. eLIFE. DOI: 10.7554/eLife.39643